IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re-application of
Trochon Veronique

Group art Unit : 1683

Serial N° : 10/764,628

Examiner : Marvich, M.

Filed : January 26, 2004

For : Method of inhibiting angiogenesis or invasion or formation of metastases

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir :

I, Veronique TROCHON, residing at 33 rue du Génie, 94400 Vitry sur Seine (France) ;

Declare and say :

I am citizen of France.

I have a PhD degree in Pharmacology and was graduated from the Rouen University (France) in 1998.

Since 2001, I have been employed by BioAlliance-Pharma Company as a Project manager where I developed the anti-angiogenic and anti-invasive metargidin disintegrin peptide project.

I am one of the inventors named in the instant US patent application and I am thoroughly familiar with the above-referenced patent application and the subject-matter described and claimed therein.

I am aware that the Examiner considered that many in vitro and animal models that are provided as evidence of success of treatment have not translated into successful treatment in humans.

In an effort to demonstrate credible clinical utility in human of the AMEP plasmid, a pre-clinical study of melanoma treatment was conducted to compare efficacy of the AMEP plasmid with temozolomide, a current reference treatment of metastatic melanoma in human.

The experiments described hereafter were performed under my supervision.

The effects of plasmid AMEP were compared to those of temozolomide in immunodeficient nude mice subcutaneously implanted with the human A375-S2 melanoma cell line.

Increasing doses of plasmid AMEP (50, 200 and 400 µg) were administered by intratumoural route followed by electrotransfer, and were compared either to a control group (vehicle) or to a reference treatment group (Temozolomide 50 mg/kg, oral route, during 5 consecutive days).

No significant differences in body weight and in mortality were observed between the placebo and plasmid AMEP groups. A reversible decrease in body weight was observed in the temozolomide group during the treatment period.

A marked dose-dependent tumour growth inhibition was observed in the plasmid AMEP-treated groups in comparison with the vehicle group. This dose-ranging study confirmed the optimal plasmid AMEP therapeutic dose at 200 µg.

Plasmid AMEP treatment (200 µg) was compared to placebo and temozolomide (Figure).

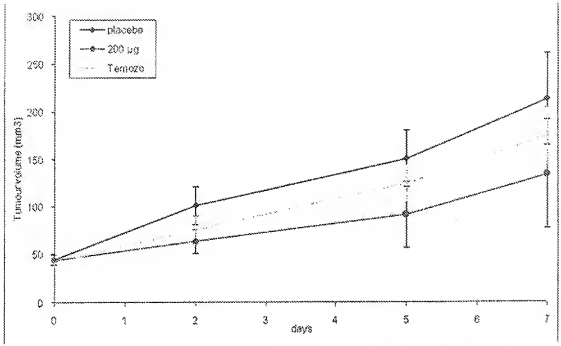

Figure : Tumour growth of A375-S2 subcutaneous tumours after intratumoural electrotransfer of increasing doses of plasmid AMEP. Increasing doses of plasmid AMEP plasmid (in 50 µl; n=11 mice/group) were injected into pre-established A375-S2 subcutaneous tumours and electric pulses were applied, when tumour volumes reached between 30 and 50 mm^3 (day 0). Data represent the tumour volume (mean ± SD) for each group.

The maximum tumour growth inhibition was observed at day 7. At 200 µg dose of AMEP plasmid, the inhibition of tumour growth reached a maximum of 36.9%. In the reference temozolomide treated group, the maximum tumour growth inhibition was 18%. The difference in tumour growth inhibition between temozolomide and the 200 µg AMEP plasmid groups was statistically significant at days 2 and 5 ($p < 0.05$). It was not significant at Day 7 despite large numerical difference because of variability.

The single intratumoural electrotransfer of plasmid AMEP inhibited the human A375-S2 melanoma tumour growth in nude mice, and was more effective

than the reference temozolomide treatment. Furthermore, plasmid AMEP treatment was better tolerated than temozolomide as no impact on body weight was recorded.

Altogether, this pre-clinical study of melanoma treatment demonstrates that the plasmid AMEP shows better efficacy and tolerance than temozolomide, which is a current reference in the treatment of metastatic melanoma in human.

Noteworthy, Health Authorities have concurred to the view that successful treatment with the plasmid AMEP can be expected in humans since a phase I clinical trial of advanced and metastatic melanoma is ongoing in Denmark and has been recently authorised in Slovenia.

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The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 02 day of July 2010